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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**ATTN: BOX MISSING PARTS**
Teruaki SEKINE et al.	:	Docket No. 2001_1248A
Serial No. 09/944,360	:	**Confirmation No. 1329**
Filed September 4, 2001	:	

METHOD AND ACTIVATED LYMPHOCYTE PREPARATIONS FOR PREVENTING RECURRENCE OF CARCINOMA

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents,
Washington, D.C.

Sir:

Kindly amend the application as follows:

IN THE SPECIFICATION

Page 4, please rewrite the paragraph beginning at [0017] as follows:

[0017] In such a case, it is preferable to collect the lymphocyte cells of more than 1×10^9 per milliliter, resulting in an improvement in efficacy of preventing recurrence of cancer. The peripheral blood is preferably collected from a vein. A desirable amount of blood collected in one operation is on the order of 0.01 to 100 ml, but it is not specifically limited. However, in order to alleviate a physical burden on a donor in collecting the blood and facilitate labor of collecting the blood and segregating lymphocyte cells from the blood, it is desirable to collect about 5 to 50 ml of peripheral blood in one operation, more preferably 10 to 20 ml of blood. In collecting the blood, heparin or citric acid may be added to the collected blood so as to prevent the collected blood from coagulating.

The segregation of the lymphocyte cells from the peripheral blood thus collected may be fulfilled by a common segregating method for lymphocyte cells such as a discontinue density gradient centrifugation using sucrose, a lymphocyte separator available on the market or other agents.

Page 6, please rewrite the paragraph beginning at [0024] as follows:

[0024] The cultivation in the invention can be fulfilled by an ordinary cell cultivating method. For instance, the cultivation may be practiced in a CO_2-incubator. It is desirable to carry out the cultivation at the CO_2-concentration of 1 to 10%, preferably 5%, at temperatures of 30°C to 40°C, preferably about 37°C.

Page 9, please rewrite the paragraph beginning at [0032] as follows:

[0032] In each tube, 44 ml of culture medium (RPMI1640+7 made by Nikken Bio Medical Laboratory Inc.) containing 35,000 U/ml of IL-2 (made by Cetus Corporation) and 5 ml of human serum were added to 50 ml of culture medium (often abbreviated as "medium") and thoroughly mixed by repeatedly turning the tube upside down to obtain cell suspension. Then, the cell suspension was put by 10 µl into tubes (Product 72,690 imported and sold by K.K. Asist) and mixed with 40 µl of Turk's solution (Made by Muto Kagaku Yakuhin) in each tube. The mixtures thus obtained were applied by 10 µl to a hemocytometer (Product No. 9731 made by Perkin-Elmer Corporation) and measured to count the number of cells under a microscope (Model 211320 made by Olympus Optical Co., Ltd.) There were obtained the results that the total numbers of the cells in the tubes were in the range of 1.0×10^7 to 7.0×10^7.

Page 9, please rewrite the paragraph beginning at [0033] as follows:

[0033] A solution consisting of 5 μl of OKT3 (imported and sold by Janssen-Kyowa Co., Ltd. and produced by Ortho Pharmaceutical) prepared previously with 8 μl of PBS(-) was poured by 10 μl into a cultivating flask having the base area of 225 cm^2 (MS-2080R made by Sumitomo Bakelite Company Ltd.) so as to uniformly soak the bottom of the flask in the solution. OKT3 in the flask was sucked out by an evacuator on the next day. Then, upon pouring 50 ml of PSB(-) into the flask, the flask kept covered was vehemently shaken, and thereafter, opened to take out the solution. Again, 50 ml of OKT3 was added into the flask, and then, upon covering the flask with the lid, the flask was vehemently shaken. Thereafter, the flask was opened and remaining liquid contents were courteously removed from the flask and lid, thus to prepare a flask containing solid-phase OKT3.

Page 10, please rewrite the paragraph beginning at [0034] as follows:

[0034] To the flask containing solid-phase OKT3 prepared in the "flask arrangement" process described above, 50 ml of cell suspension obtained in the aforesaid "lymphocyte segregation" process was distributed. Then, cultivation in the flask was performed at 37°C in the presence of carbon dioxide gas having a concentration of 5%. After five days, 50 ml of culture medium was added, and the cultivation was continued at 37°C in the presence of carbon dioxide gas having a concentration of 5%. After the following four days, 150 ml of culture medium was added, and further cultivation was performed at 37°C in the presence of carbon dioxide gas having a concentration of 5%. For two more days, the cultivation was continued at 37°C in the presence of carbon dioxide gas having a concentration of 5%. Consequently, 2.0×10^8 to 7.0×10^8 of activated lymphocytes could be obtained.

Page 12, please rewrite the paragraph beginning at [0038] as follows:

[0038] To the aforesaid cell pellets, 200 ml of physiological saline solution containing 2% of human albumin were added to allow the cell pellets to be suspended therein. Lastly, the desired preparations to be administered to a cancer patient were prepared by filtrating the solution through a stainless wire filter of 100 μ in mesh and packing it into a blood transfusion bag. In this case, the number of cells packed in the blood transfusion bag was 6×10^9 to 20×10^9.

Page 13, please rewrite the paragraph beginning at [0041] as follows:

[0041] Next, one example of cryopreserving the activated lymphocytes prepared in the aforementioned "production" process will be described. Upon centrifuging the activated lymphocytes obtained in the "production" process, the culture medium is removed by decantation to obtain cell pellets. To the cell pellets, 18 ml of cell preserving solution (prepared by mixing 5 ml of human serum, dimethyl sulfoxide (made by Nacalai Tesque, Inc., hereinafter abbreviated as "DMSO") with 40 ml of culture medium (RPMI1640+7)) is added. After fully mixing the mixture thus obtained, it is distributed by 3 ml into five cell preserving tubes of 5 ml in capacity (imported and sold by Corning International) (5×10^7 per tube). The cell preserving tubes thus prepared are placed in a superthermal freezer and preserved at -80°C.

IN THE CLAIMS

Kindly amend the claims as follows:

12. **(Amended)** The method for preventing recurrence of cancer for a long period of time set forth in claim 1, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.

13. **(Amended)** The method for preventing recurrence of cancer for a long period of time set forth in claim 3, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.

14. **(Amended)** The method for preventing recurrence of cancer for a long period of time set forth in claim 7, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.

15. **(Amended)** The method for preventing recurrence of cancer for a long period of time set forth in claim 9, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.

REMARKS

The foregoing amendments are presented to correct minor clerical errors which are apparent.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**<u>Version with markings to show changes made</u>**".

Favorable action on the merits is solicited.

Respectfully submitted,

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[0014] The method of the invention is practiced while carrying out proceedings of treating cancer, specifically liver cancer and preventing recurrence of cancer with administration of activated lymphocytes, so that a complete therapeutic effect can be attained to prolong the effect of preventing recurrence of cancer for a long period of at least five or more years. In concrete terms, the present invention is featured in that the treatment of cancer according the invention is performed along with administration of the preparations made from the activated lymphocytes of the invention.

[0015] The "treatment of cancer" as termed herein has principally a meaning of a surgical operation. Thus, the efficacy of preventing recurrence of cancer for a long period of at least five or more years can be secured by administering the activated lymphocytes to the cancer patient several times. The treatment of cancer may imply not only the surgical operation noted above, but also chemotherapeutic treatment and radiotherapy.

[Collecting of lymphocyte cells]

[0016] The collection of lymphocyte cells is carried out when need arises in principle. The lymphocyte cells are prepared by segregating lymphocytes from the peripheral blood of a cancer patient himself to be treated or the other cancer patient than the cancer patient to be treated.

[0017] In such a case, it is preferable to collect the lymphocyte cells of more than 1×10^9 per milliliter, resulting in an improvement in efficacy of preventing recurrence of cancer. The peripheral blood is preferably collected from a vein. A desirable amount of blood collected in one operation is on the order of 0.01 to 100 ml, but it is not specifically limited. However, in order to alleviate a physical burden on a donor in collecting the blood and facilitate labor of collecting the blood and segregating lymphocyte cells from the blood, it is desirable to collect about 5 to 50 ml of peripheral blood in one operation, more preferably 10 to 20 ml of blood. In collecting the blood, heparin or citric acid may be added to the collected blood so as to prevent the collected blood from coagulating. The segregation of the lymphocyte cells from the

peripheral blood thus collected may be fulfilled by a common segregating method for lymphocyte cells such as a discontinue density gradient centrifugation using sucrose, a lymphocyte separator available on the market or other agents.

[Proliferation of Lymphocyte cells]

[0018] Next, proliferation and cultivation of cells collected in the manner as noted above will be described. The proliferation of the lymphocyte cells according to the invention is carried out by proliferating cultivation or activating cultivation in the presence of a combination of solid-phase anti-CD3 antigen and interleukin 2. In the cultivation, the lymphocyte cells may be suspended in a culture medium containing interleukin 2 to be cultivated in a culture in a culture kit with addition of solid-phase anti-CD3 antigen. In this case, various types of mitogen growth factors or activating factors may be used in proliferating and activating the cells at need.

[0019] Any type of anti-CD3 antigen capable of hastening the proliferation and activation of the lymphocyte cells may be used. That is, the type of the anti-CD3 antigen is not specifically limited in the invention. The anti-CD3 antigen used for stimulating the lymphocyte cells may be yielded in an animal or cells by use of refined CD3 molecules, or there may be advantageously used commercial OKT-3 antigen (produced by Ortho Pharmaceutical) from the viewpoint of cost and stability.

[0020] It is desirable to use the anti-CD3 antigen in its solid phase in consideration in the light of the efficiency of proliferating and handling the lymphocyte cells. There may be used implements made of glass, polyurethane, polyolefine, polystyrene or the like for solidifying the antigen, or a sterilized cell-cultivating plastic flask, which is easy to obtain at the market. The size of the flask may be arbitrarily determined.

[0021] It is desirable to prepare the anti-CD3 antigen by placing dilute solution of anti-CD3 antigen into a solidifying implement and permitting it to stand in its solid phase at temperatures of, for example, 4℃ to 37℃ for 2 to

24 hours. In solidifying, the anti-CD3 antigen may be preferably diluted with physiological saline solution such as sterized Dulbecco's phosphate buffered saline solution into concentrations of 1 to 30 μ g/ml. After solidifying, the anti-CD3 antigen may be preserved in a cold room or refrigerator (4℃) until use. When used, the physiological saline solution is removed from the anti-CD3 antigen, and the anti-CD3 antigen may be rinsed out with Dulbecco's phosphate buffered saline solution at normal temperature, as occasion demands.

[0022] It is preferable to put interleukin 2 into the culture solution to improve the efficiency of proliferation. The commercial interleukin 2 available on the market may be desirably used in concentrations of from 1 to 2000 U/ml. The interleukin 2 may be dissolved in a culture solution for cultivating cells, which has been widely used in this field of art, such as water, physiological saline solution, Dulbecco's phosphate buffered saline solution, RPMI-1640, DMEM, IMDM and AIM-V. The interleukin 2 once dissolved may be preferably cryopreserved to prevent deterioration of activity thereof.

[0023] In this case, any type of culture solution suitable for cultivation of the lymphocyte cells may be used, and therefore, the type of the culture solution is not specifically limited in the invention. That is, there may be suitably used a culture solution derived from an organism, such as serum, and a synthetic medium prepared by adding amino acid, vitamin, uncleic acid base or the like to an equilibrium saline solution. As the suitable culture solution in this case, there may be enumerated, for example, RPMI-1640, AIM-V, DMEM and IMDM. In specific, of these solutions, RPMI-1640 is most suitable. It is desirable to add normal human serum to the culture medium, resulting in producing an excellent effect of proliferating the lymphocytes. The culture medium available at the market may be used.

[0024] The cultivation in the invention can be fulfilled by an ordinary cell cultivating method. For instance, the cultivation may be practiced in a CO_2-incubator. It is desirable to carry out the cultivation at the CO_2-

concentration of 1 to 10%, preferably 5%, at temperatures of 30℃ to 40℃, preferably about 37℃.

[Administration of Activated lymphocytes]

[0025] The more the frequency of administering the activated lymphocytes is, the higher the therapeutic efficacy becomes. However, the administration of the activated lymphocytes to a cancer patient is generally made every several days to several months. The administration of the activated lymphocytes may commence not only after conducting an operation of surgical removal, chemotherapeutic treatment or radiotherapy, but also at the earliest possible time before taking surgical or chemotherapeutic treatment or radiotherapy, so that the efficacy of preventing recurrence of cancer can be more improved. It is desirable to continue administering the activated lymphocytes to the cancer patient over about five years after the operation, if possible. However, in general, even short term administration of the activated lymphocytes for six months to eight months after the operation can securely produce a sufficient effect of preventing recurrence of cancer for the following five or more years.

[0026] In general, the administration of the activated lymphocytes may possibly be made 1 to 1000 times. However, the administration made once has relatively little effect. Thus, in order to stably produce the stable effect of preventing recurrence of cancer for five or more years after conducting the operation of treating the cancer, it is required to administer the activated lymphocytes to the patient in therapy at least five or more times within eight years after the operation for the cancer. That is, the more the frequency of administering the activated lymphocytes to the patient is, the higher the effect of preventing recurrence of cancer becomes. In either way, the conditions of the frequency of administering the activated lymphocytes, the period of time within which the activated lymphocytes are administered, and the number of times the administration is made may be arbitrarily determined according to circumstances.

[0027] In the method of the invention, the administration of the activated

Thereafter, the blood collected in each centrifugal settler tube was diluted three times with a culture solution to obtain equivalently diluted blood.

[0030] Thereafter, each of the centrifugal settler tubes was tightly covered with a lid, and then, turned upside down several times to mingle. Then, into six centrifugal settler tubes each of 15 ml in volume, 15 ml of Lymphosepar-I (made by Immuno-Biological Laboratories Co., Ltd.) were transfused by using a pipette of 10 ml in capacity (Pipette 4105 imported and sold by Corning International). Then, 10 ml of blood diluted with the rinsing culture medium were slowly poured into the respective centrifugal settler tubes, so as not to disturb the surface of the solution in each tube and thereafter, centrifuged at a relatively low speed of 1800 rpm at a centrifugal separating temperature of 20℃ for 15 minutes with a centrifugal separator with its brakes off. (A centrifugal settler H-700R made by Kokusan Co. Ltd. was used.)

[0031] After centrifugal sedimentation, the supernatant liquid of the liquid contents centrifuged in each of the tubes was slowly sucked up to a depth of about 1 cm above the lymphocyte layer centrifugally precipitated in each tube in an aseptic condition by using an aspirator so as not to suck in the lymphocyte cells. Then, the layer of the lymphocyte cells in the tube was sucked up by using a pipette of 5 ml in capacity so as not to suck in blood clots and collected with a centrifugal settler tube of 50 ml in capacity, in which 25 ml of rinsing culture medium (RPMI1640+6) were contained in advance. Then, the centrifugal settler tube was covered with a lid and turned upside down several times to mingle. Thereafter, the centrifugal settler tube was centrifuged at 1800 rpm at a centrifugal separating temperature of 20℃ for 10 minutes. After each of the centrifugal settler tube was further centrifuged, the supernatant liquid in the tube was removed and thoroughly dispersed by using a vortex mixer.

[0032] In each tube, 44 ml of culture medium (RPMI1640+7 made by Nikken Bio Medical Laboratory Inc.) containing 35,000 U/ml of IL-2 (made by Cetus Corporation) and 5 ml of human serum were added to 50 ml of culture

medium (often abbreviated as "medium") and thoroughly mixed by repeatedly turning the tube upside down to obtain cell suspension. Then, the cell suspension was put by 10 μl into tubes (Product 72.690 imported and sold by K.K. Asist) and mixed with 40 μl of Turk's solution (made by Muto Kagaku Yakuhin) in each tube. The mixtures thus obtained were applied by 10 μl to a hemocytometer (Product No. 9731 made by Perkin-Elmer Corporation) and measured to count the number of cells under a microscope (Model 211320 made by Olympus Optical Co., Ltd.) There were obtained the results that the total numbers of the cells in the tubes were in the range of ~~1.0×10⁷ to 7.0×10⁷~~ 1.0×10^{4} to 7.0×10^{7}.

[Arrangement of flask containing solid-phase OKT3]

[0033] A solution consisting of 5 μl of OKT3 (imported and sold by Janssen-Kyowa Co., Ltd. and produced by Ortho Pharmaceutical) prepared previously with 8 μl of PBS(-) was poured by 10 μl into a cultivating flask having the base area of 225 cm^2 (MS-2080R made by Sumitomo Bakelite Company Ltd.) so as to uniformly soak the bottom of the flask in the solution. OKT3 in the flask was sucked out by an evacuator on the next day. Then, Upon pouring 50 ml of PSB(-) into the flask, the flask kept covered was vehemently shaken, and thereafter, opened to take out the solution. Again, 50 ml of OKT3 was added into the flask, and then, upon covering the flask with the lid, the flask was vehemently shaken. Thereafter, the flask was opened and remaining liquid contents were courteously removed from the flask and lid, thus to prepare a flask containing solid-phase OKT3.

[Cultivation for activating lymphocytes]

[0034] To the flask containing solid-phase OKT3 prepared in the "flask arrangement" process described above, 50 ml of cell suspension obtained in the aforesaid "lymphocyte segregation" process was distributed. Then, cultivation in the flask was performed at 37℃ in the presence of carbon dioxide gas having a concentration of 5%. After five days, 50 ml of culture medium was added, and the cultivation was continued at 37℃ in the

presence of carbon dioxide gas having a concentration of 5%. After the following four days, 150 ml of culture medium was added, and further cultivation was performed at 37℃ in the presence of carbon dioxide gas having a concentration of 5%. For two more days, the cultivation was continued at 37 ℃ in the presence of carbon dioxide gas having a concentration of 5%. Consequently, ~~2.0 × 108 to 7.0 × 108~~ 2.0×10⁸ to 7.0×10⁸ of activated lymphocytes could be obtained.

[Cultivation for growing lymphocytes]

[0035] The lymphocytes prepared in the aforementioned "lymphocyte activating cultivation" process was transfused to a cultivating gas-permeable bag containing 750 ml of culture medium LL-7 (made by Nikken Bio Medical Laboratory Inc.) or Medium 930 (made by Kohjin Bio Co. Ltd.) and cultivated at 37℃ in the presence of carbon dioxide gas having a concentration of 5% in a carbon dioxide incubator (Model CDP-300A made by K.K. Hirasawa). After two days, the cultivating gas-permeable bag ("Nipro Culture Bag A-1000" made by Nipro Medical Corporation) containing the cells and another cultivating gas-permeable bag containing new culture medium were joined to each other by a germfree connector (made by Terumo Corporation). Upon fully mixing the culture medium in the joined gas-permeable bags, the joined bags were cut apart, and the cut portions of the bags were aseptically sealed. Thereafter, cultivation was performed at 37℃ in the presence of carbon dioxide gas having a concentration of 5%.

[0036] After two more days, the lymphocyte cells were cultivated by using two gas-permeable bags in which the cultivation was continued and two other gas-permeable bags each containing new culture medium having the cells dispersed uniformly. That is, the cultivation was carried out by using the four gas-permeable bags. After the following two days, cultivation was performed by using the aforesaid four gas-permeable bags containing the cells and two new gas-permeable bags having the cells dispersed uniformly therein.

[Production of preparations to be administered]

[0037] The culture mediums containing the cells in three to six of the aforementioned gas-permeable bags were transfused into 250 ml centrifuge tubes (made by Corning International) to centrifugally segregate the cells. Then, rinsing of the cells was carried out by removing the culture solutions from the tubes by decantation, adding physiological saline solutions to cell pellets to suspend the cells in the solutions, and subjecting the cells to centrifugal segregation. Further, the same rinsing was carried out by physiological saline solution containing 0.1% of human albumin instead of the aforesaid physiological saline solution, thus to prepare cell pellets.

[0038] To the aforesaid cell pellets, 200 ml of physiological saline solution containing 2% of human albumin were added to allow the cell pellets to be suspended therein. Lastly, the desired preparations to be administered to a cancer patient were prepared by filtrating the solution through a stainless wire filter of 100 μ in mesh and packing it into a blood transfusion bag. In this case, the number of cells packed in the blood transfusion bag was 6×10^9 to 20×10^9.

[Administration of the preparations]

[0039] The preparations produced in the aforesaid "production" process were inoculated into the vein of the liver cancer patient from which the blood was drawn in the "segregation" process described above. The administration of the preparations was performed three times within three weeks after performing an operation for the liver cancer, and after three and six months from the operation for the cancer. Namely, the preparations according to the invention were administered to the patient of liver cancer five times in total.

[Judgment of the effect of preventing recurrence of cancer]

[0040] An analysis to determine the rate of recurrence of cancer from the commencement of the treatment until five years after the operation for cancer was performed on forty-nine cases dosed with the activated lymphocytes according to the invention and fifty-two cases who did not dosed with the

activated lymphocytes. It was evident from the result of the analysis that the relapse-free survival of the latter (cases dosed with no activated lymphocyte) was 21%, but the relapse-free survival of the former (cases dosed with the activated lymphocytes of the invention) was 35%. Thus, there could be recognized a significant difference therebetween based on a probability level of 1%. Of the cases dosed with the activated lymphocytes of the invention, no case was caught by serious harmful side effects, and some cases became slightly feverish. That is, the result of the analysis reveals that the activated lymphocytes according to the invention have the excellent efficacy of preventing recurrence of cancer (in particular, liver cancer) for a long period of at least five or more years, and besides, the activated lymphocytes of the invention has very little side effects.

[0041] Next, one example of cryopreserving the activated lymphocytes prepared in the aforementioned "production" process will be described. Upon centrifuging the activated lymphocytes obtained in the "production" process, the culture medium is removed by decantation to obtain cell pellets. To the cell pellets, 18 ml of cell preserving solution (prepared by mixing 5 ml of human serum, dimethyl sulfoxide (made by Nacalai Tesque, Inc., hereinafter abbreviated as "DMSO") with 40 ml of culture medium (RPMI1640+7)) is added. After fully mixing the mixture thus obtained, it is distributed by 3 ml into five cell preserving tubes of 5 ml in capacity (imported and sold by Corning International) (5×10^7 per tube). The cell preserving tubes thus prepared are placed in a superthermal freezer and preserved at -80℃.

[0042] In thawing the frozen lymphocyte cells, the tubes are taken out from the freezer and warmed with a heat block (Model TAL-1G made by Tietech Co. Ltd.) at 37℃ for four minutes to thaw and restore the frozen lymphocyte cells. The lymphocyte cells thus thawed and restored are aseptically transfused by 3 ml into a centrifugal settler tubes of volume 50 ml, and 10 ml of physiological saline solution is added thereto to produce suspension. After centrifuging the suspension (at 1000 rpm at 20℃ for 5 minutes), supernatant

10. The method for preventing recurrence of cancer for a long period of time set forth in claim 3, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need.

11. The method for preventing recurrence of cancer for a long period of time set forth in claim 7, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need.

(Amended)
12. The method for preventing recurrence of cancer for a long period of time set forth in claim 1, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.

(Amended)
13. The method for preventing recurrence of cancer for a long period of time set forth in claim 3, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^9 per milliliter.

(Amended)

14. The method for preventing recurrence of cancer for a long period of time set forth in claim 7, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^{9} per milliliter.

(Amended)

15. The method for preventing recurrence of cancer for a long period of time set forth in claim 9, wherein said activated lymphocytes to be administered while performing treatment of cancer are prepared from a cancer patient or the other cancer patient at need, said lymphocytes having cells of more than 1×10^{9} per milliliter.